Expanding the genetic and phenotypic spectrum of *CHD2*-related disease: from early neurodevelopmental disorders to adult-onset epilepsy.

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Abstract

CHD2 encodes the chromodomain helicase DNA-binding protein 2, an ATP-dependent enzyme that acts as a chromatin remodeler. *CHD2* pathogenic variants have been associated with various early onset phenotypes including developmental and epileptic encephalopathy (DEE), self-limiting or pharmacoresponsive epilepsies and neurodevelopmental disorders without epilepsy. We reviewed 84 previously reported patients carrying 76 different *CHD2* pathogenic or likely pathogenic variants and describe 18 unreported patients carrying 12 novel pathogenic or likely pathogenic variants, two recurrent likely pathogenic variants (in two patients each), three previously reported pathogenic variants, one gross deletion. We also describe a novel phenotype of adult-onset pharmacoresistant epilepsy, associated with a novel *CHD2* missense likely pathogenic variant, located in an interdomain region. A combined review of previously published and our own observations indicates that although most patients (72.5%) carry truncating *CHD2* pathogenic variants, *CHD2*-related phenotypes encompass a wide spectrum of conditions with developmental delay/intellectual disability, including prominent language impairment, attention deficit hyperactivity disorder (ADHD) and autistic spectrum disorder (ASD). Epilepsy is present in 92% of patients with a median age of seizure onset of 2 years and 6 months. Generalised epilepsy types are prevalent and account for 75.5% of all epilepsies, with photosensitivity being a common feature and adult-onset non-syndromic epilepsy a rare presentation. No clear genotype-phenotype correlation has emerged.

Keywords: *CHD2*, chromatin-remodelling enzymes, genetic epilepsy, neurodevelopmental disorders

Introduction

Chromatin dynamic organisation is necessary to allow or prohibit access to genes by transcription mechanisms and consequently to indirectly activate or inhibit gene transcription. Chromatin remodelers are versatile components of the transcriptional apparatus and are necessary to catalyse the broad range of chromatin changing reactions (Hall and Georgel, 2007).

Chromodomain helicase DNA-binding (CHD) proteins are a family of ATP-dependent chromatin remodelers which belong to the SNF2 superfamily (Bork and Koonin, 1993; Eisen et al., 1995). Their structure is highly conserved from yeast to humans. They are characterised by two N-terminal chromodomains that function as interaction surfaces for a variety of chromatin components and distinguish CHD proteins from other SNF2 superfamily members (Delmas et al., 1993; Woodage et al 1997). Nine members have been identified in humans (CHD1–CHD9) and their role in determining human disease was first described in patients with coloboma, heart defects, atresia choanae, growth retardation, genital and ear abnormalities (CHARGE syndrome) carrying *CHD7* pathogenic variants (Vissers et al., 2004).

CHD2 is the only member of CHD-protein family where its gene pathogenic variants produce a brain-restricted phenotype (Lamar and Carvill, 2018). The *CHD2* gene (MIM# 602119), located on chromosome 15q26.1, encodes the chromodomain helicase DNA-binding protein 2 and has a mRNA transcript of 9374 bp sequence (NM_001271.3) containing 39 exons. CHD2 expression is ubiquitous with the highest levels in adult human tissue in the thyroid, lung, prostate, brain, adrenal gland, testis, ovary, lymph node (https://www.gtexportal.org/; http://www.ensembl.info/2011/05/24/human-bodymap-2-0-data-from-illumina/).

CHD2 pathogenic variants have been associated with various neurological phenotypes, with developmental and epileptic encephalopathy (DEE) being the most common. Self-limiting or pharmacoresponsive epilepsies and neurodevelopmental disorders without epilepsy are additional clinical presentations.

Li et al. (2008) described a chromosomal translocation t(15;22) (q26.1;q11.2) and a 3.3 Mb deletion adjacent to the 15q26 breakpoint where the *CHD2* gene is located, in an adopted child with developmental delay and two episodes of febrile seizures. Veredice et al. (2009) described a 30-month-old girl with DEE and an interstitial deletion of 5 Mb affecting the 15q26.1–26.2 chromosomal region, including the *CHD2* gene. After these initial descriptions, several *CHD2* deletions, duplications, insertions, and pathogenic variants have been detected in cohorts of individuals with DEE, epilepsy and/or neurodevelopmental disorders, particularly intellectual disability, and autism.

Here, we provide a literature review of 84 previously published patients and expand the genetic and phenotypic spectrum of *CHD2*-related disease with 18 unreported patients carrying 12 novel pathogenic variants, of which two recurrent, three previously reported pathogenic variants and one gross deletion.

Material and methods

We found 12 novel *CHD2* pathogenic or likely pathogenic variants from a heterogeneous cohort of 18 previously unreported patients with epilepsy onset in childhood (n=17) or adulthood (n=1). We retrospectively reviewed clinical, video-EEG, neuroimaging, neuropsychological and genetic information of all patients included in our cohort, and useda standardised template to collate and analyse the data. EEG were available for all patients and MRI for 88% (n=16/18) in our cohort. We classified seizure and epilepsy types and syndromes according to the ILAE criteria (Fisher et al., 2017; Scheffer et al., 2017). All patients were genetically tested at the Anna Meyer Children's Hospital (Florence, Italy) and nine of them were followed clinically at the same centre while the remaining patients referred to other Italian tertiary centres. The study was approved by the Paediatric Ethics Committee of the Tuscany Region and informed consent was obtained by patients, parents or guardians.

We extracted DNA from peripheral blood leukocytes using a QiaSymphony SP robot (Qiagen, Hilden, Germany) according to the manufacturer's protocol. We quantified high-quality DNA using a Quantifluor Fluorometer (Promega, Madison, WI, USA). We performed Targeted resequencing of a panel including 159 genes associated with epilepsy (full list provided in Supplementary Table 1) in 17 patients and Whole Exome Sequencing in one patient. We performed target enrichment and library preparation using a custom designed Nextera Rapid Capture assay (Illumina, San Diego, CA, USA) and we used an Illumina NextSeq550 (Illumina, San Diego, CA, USA) for sequencing. We aligned reads to human genome build GRCh37/UCSC hg19. We quality-filtered variants according to GATK's best practices, annotated with VarSeq (Golden Helix, USA), and filtered against public (gnomAD V.2.0) and in-house databases to retain private and rare (MAF <0.1%) variants located in exons with any effect on the coding sequence, and within splice site regions. We filtered variants with depth >10 and genotype quality >20 according to the de novo autosomal dominant, X-linked, homozygous recessive and compound heterozygous models. We evaluated the functional impact of variants through in silico prediction using the dbNSFP database (v3.3a) (Liu et al., 2016). We obtained variant validation and segregation with Sanger sequencing on both strands, using the Big Dye Terminator V3.1 chemistry (Thermo Fisher, MA, USA), on a 3500 DX Genetic

Analyzer (Thermo Fisher, MA, USA). We classified variants according to the international guidelines of the ACMG Laboratory Practice Committee Working Group (Richards et al., 2015).

We reviewed the literature for previously published data on *CHD2* pathogenic variants. In the PubMed database, we searched for publications including *CHD2* pathogenic variants by using as MeSH terms "CHD2" and either "mutations" or "variants", within the time frame ranging from the first published patient with epilepsy (Veredice et al., 2009) until April 2021. The reference lists of the selected publications were checked for further potentially suitable publications. The pathogenic variants identified in the selected publications were cross checked with the *CHD2* damaging or potentially damaging variants listed in Human Gene Mutation Database [HGMD] and data on the associated neurological phenotype were extracted, when available.

We included 84 previously reported patients harbouring 76 different *CHD2* pathogenic or likely pathogenic variants, with a stepwise approach, including screening of genetic data followed by review of available phenotypic information (Figure 1).

Genetic inclusion criteria were gross deletions or duplications involving the *CHD2* gene, and missense or truncating variants classified as pathogenic or likely pathogenic according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015) (Li et al., 2008; Veredice et al., 2009; Dhamija et al., 2011; Rauch et al., 2012; Allen et al., 2013; Lund et al., 2013, 2014; Suls et al., 2013; Carvill et al., 2013; Courage et al., 2014; Hamdan et al., 2014; Appenzeller et al., 2014; O'Roak et al., 2014; Pinto et al., 2014; Chénier et al., 2014; Fitzgerald et al., 2015; Galizia et al., 2015; Thomas et al., 2015; Trivisano et al., 2015; Verhoeven et al., 2016; Lebrun et al., 2017; Bernardo et al., 2017; Wang et al., 2017b, 2017a, 2019; Liu et al., 2018; Monlong et al., 2018; Petersen et al., 2018; Rim et al., 2018; Zhou et al., 2018; Caputo et al., 2018; Angione et al., 2019; Demos et al., 2019; Jiao et al., 2019; Peng et al., 2019; Routier et al., 2019; Snoeijen-Schouwenaars et al., 2019; Symonds et al., 2019; Tsang et al., 2019; Costain et al., 2019; Poisson et al., 2020; Cabrera-Salcedo et al., 2020; Ziats et al., 2020; Chen et al., 2020).

We excluded those patients harbouring variants of uncertain significance (VOUS) (Galizia et al., 2015; Wang et al., 2016; Ortega-Moreno et al., 2017; Trujillano et al., 2017; Fernández-Marmiesse et al., 2019). We also excluded one paper not reporting the exact *CHD2* variant (Singh and Ritaccio, 2020).

Of the patients with pathogenic or likely pathogenic meeting these genetic criteria (n=103), we included those with available information on neurological phenotype, including either the epilepsy type (Scheffer et al, 2017) (n=76) or, for those without epilepsy, at least two of the following clinical items: motor development, speech development, cognitive functioning, presence/absence of attention deficit/hyperactivity, deficits in communication, social and learning skills (n=8) (Li et al.,

2008; Chénier et al., 2014; Hamdan et al., 2014; O'Roak et al., 2014; Pinto et al., 2014; Lebrun et al., 2017; Jiao et al., 2019; Cabrera-Salcedo et al., 2020). We did not have access to the original data from patients published in the previous studies, and we included information on EEG, neuroimaging and cognition when available.

Results

Genotypic Spectrum

In the 18 novel patients we are describing, we identified one gross deletion and 15 different *CHD2* missense (n=5) or truncating variants (n=10) that we classified as pathogenic or likely pathogenic according to the ACMG criteria (Figure 1 and Table 1). Twelve pathogenic or likely pathogenic variants are newly reported here and three were previously described (Wang et al., 2017b, 2017a; Strauss et al., 2018; Ziats et al., 2020). Among the 12 novel pathogenic or likely pathogenic variants, two were recurrent in two patients each, the p.Arg699Trp (#85 and #92) and the p.Arg900Gly (#86 and #96). Except for two frameshift pathogenic variants for which segregation analysis could not be performed, all remaining pathogenic or likely pathogenic variants occurred *de novo* (Table 1). All the five missense likely pathogenic variants occurred at highly conserved amino acid residues and were located in functional domains, with the exception of the p.Trp1261Ser (#89) variant falling in an interdomain region (Figure 2). All pathogenic or likely pathogenic variants were predicted to be damaging by *in silico* prediction models (https://varsome.com/variant/hg19/) and were absent in the gnomAD database.

Among 102 new and previously published patients, the majority (72.5%, n = 74/102) carried truncating *CHD2* pathogenic variants (25 frameshift, 21 nonsense, 17 deletions/duplications and 4 splicing).

Frameshift and nonsense pathogenic variants introduce a premature stop codon in the mRNA and are predicted to elicit a rapid degradation of the CHD2 mRNA since they activate the nonsense-mediated mRNA decay (NMD). None of the *CHD2* frameshift and nonsense pathogenic variants in our series occurred in the 3'-most exon or within the 3'-most 50 nucleotides of the penultimate exon. Generally, NMD is not predicted to occur if the premature termination codon falls in these regions (Chang, Imam, & Wilkinson, 2007; Lewis, Green, & Brenner, 2003). This observation further confirms the haploinsufficiency mechanism of *CHD2* pathogenic variants.

Overall, 24 missense pathogenic or likely pathogenic variants were identified in 28 patients (30.6%, n = 28/102), and 16/24 of these were in functional domains, mainly in the two helicase domains. No missense pathogenic or likely pathogenic variants were in the chromodomains. The chromodomains, the helicase domains and the putative DNA-binding domain are depleted of

missense variation in the general population as indicated by missense variants present more than twice in the GnomAD database (Figure 2). The remaining eight missense pathogenic or likely pathogenic variants were in the interdomain regions.

Using the splice site prediction tools available on the Alamut Visual platform (https://www.interactive-biosoftware.com/alamut-visual/), we did not observe significant splicing alteration for any of the 24 missense pathogenic or likely pathogenic variants.

Phenotypic Spectrum

Clinical and variant data of the 102 patients (84 previously reported and 18 novel) are presented in Table 1 and Supplementary Table 2. There were 55 males and 42 females (gender ratio M/F = 1.3/1; data available for n = 97/102), with an age at description ranging from 17 months to 38 years (median age at description of 11 years; data available for n = 87/102).

Epilepsy was present in 94/102 patients (92.2%) with a median age at seizure onset of two years and six months; range 3 months (#82) - 22 years (#89) (data available for n = 87/94). Of the eight patients (7.8%) who did not have a diagnosis of epilepsy, six never had any seizures (#17, #19, #21, #24, #41, #64) and two experienced febrile seizures (#2, #58). The mean age of patients without epilepsy was 8 years (median 9 years, range 5-15 years, data available for n 7/8).

Fifty-seven patients with epilepsy manifested multiple seizure types, including generalised onset tonic–clonic seizures (n = 47), absences (n = 38), myoclonic seizures (n = 37), focal-onset seizures (n = 21), atonic seizures (n = 18), myoclonic-atonic seizures (n = 13), tonic seizures (n = 11), epileptic spasms (n = 2), myoclonic-clonic (n = 1). Eleven of these patients also had a history of febrile seizures (n = 11). Thirty-three patients manifested a single seizure type, including generalised onset tonic-clonic seizures (n = 12), absences (n = 13), focal seizures (n = 2), myoclonic (n = 4) and myoclonic-atonic seizures (n = 2). Four of these patients also had a history of febrile seizures (n = 4). Seizure types were unknown in the four remaining patients with epilepsy.

A history of status epilepticus (SE) was reported in 18 patients, including convulsive (n=10), non-convulsive (n=6), and both convulsive and non-convulsive episodes (n=2).

The prevalent epilepsy type was generalised, occurring in 75.5% (n = 71/94), followed by combined generalised and focal in 22.3% (n = 21/94), and focal in the remaining 2.2% (n = 2/94).

Epilepsy syndromes encompassed a wide range of presentations including epilepsy with myoclonic-atonic seizures (EMAS) (n = 10/94), epilepsy with eyelid myoclonia (EEM) (n = 9/94), other types of generalised epilepsies (GE) (n = 4/94), Lennox-Gastaut syndrome (LGS) (n = 6/94) and West syndrome (WS) (n = 1/94). One patient had self-limiting occipital lobe epilepsy and one patient

Childhood Absence Epilepsy (CAE) evolving to occipital lobe epilepsy. Two patients presented with a mild 'febrile seizures plus' phenotype.

Cognitive status was assessed with formal testing in 32/102 patients, while was only based on clinical judgement in 60 more patients and unknown in the remaining 10. Intellectual disability (ID) was reported in 82.6% of patients (data available n = 92/102, ID reported in n = 76/92): mild ID in 28.9% (n = 22/76), moderate ID in 25% (n = 19/76), severe-profound ID in 14.5% (n = 11/76), not specified degree of cognitive impairment in 31.6% (n = 24/76). The remaining 17.4% of patients (n = 16/92) had normal cognitive functions.

Other neurodevelopmental or psychiatric disorders were reported in 67.8% of patients with available data (data available n = 84/102, reported in n = 57/84) (Table 2).

Electroencephalographic (EEG) data were available for 60.3% of patients with epilepsy (n = 57/94). EEGs showed slow background activity in a minority of patients (n=19), and a combination of generalised spikes and/or spikes and waves (n = 49), focal (n = 10) or multifocal (n = 6) abnormalities. Hypsarrhythmia was reported in one patient with epileptic spasms (#82). Photosensitivity was reported in 45.6% patients with epilepsy (data available for n= 79/94, reported in 36/79): more specifically, both clinical and EEG photosensitivity was described in nine patients (Veredice et al., 2009; Chénier et al., 2014; Galizia et al., 2015; Thomas et al., 2015; Trivisano et al., 2015; Caputo et al., 2018), only clinical in eight (Carvill et al., 2013; Thomas et al., 2015), and only electrographic in two (n = 2) (Chénier et al., 2014; Poisson et al., 2020). No further details are available for the remaining patients with reported photosensitivity (n = 17).

Brain magnetic resonance imaging (MRI) was available (considering both neuroimaging reviewed directly and descriptions acquired by reports) for 79.4% patients (n = 81/102) and was reported as normal in 85.4% (n=70/82). Abnormal MRI findings included brain atrophy (4), corpus callosum hypoplasia (n = 4), cerebellar vermis hypoplasia (n = 2), cerebellar atrophy (n = 2), Arnold-Chiari type I malformation (n=2), not otherwise specified white matter hyperintensity (n=1). One patient had a normal computed tomography (CT) scan (#19).

In our own cohort, we identified a novel likely pathogenic missense variant in a patient with adult-onset focal and generalised-onset seizures, which were severe and pharmacoresistant from the onset. Prior to seizure onset, the patient had normal psychomotor development, attended school with support for dyslexia and graduated with good results. Cognitive assessment, performed at the age of 27, after seizure-onset, with Wechsler Adult Intelligence Scale – Fourth Edition (WAIS–IV), revealed mild intellectual disability (Full Scale IQ (FSIQ) = 61, Verbal Comprehension Index (VCI) = 75, Perceptual Reasoning Index (PRI) = 75, Working Memory Index (WMI) = 66,

Processing Speed Index (PSI) = 81). No behavioural or psychiatric problems were reported. Adult-onset of epilepsy has not been reported in association with *CHD2* pathogenic or likely pathogenic variants so far.

Discussion

In this study, we described 18 unreported patients carrying 12 novel *CHD2* pathogenic or likely pathogenic variants, two recurrent likely pathogenic variants (in two patients each), three previously reported pathogenic variants, one gross deletion, and reviewed 84 previously reported patients carrying 76 different *CHD2* pathogenic or likely pathogenic variants.

Despite the high number of *CHD2* pathogenic or likely pathogenic variants previously reported, no clear genotype-phenotype correlation emerged from our study.

Combining previously published patients with those newly reported here, the majority of *CHD2* pathogenic variants are truncating (72.5%, frameshift, splicing and deletions/duplications), confirming that the pathogenic mechanism of *CHD2*-associated phenotype is haploinsufficiency. Missense pathogenic or likely pathogenic variants are in either functional domains or interdomain regions without specific mutation hotspots. Overall, variants location in the protein does not seem to cause a direct effect on phenotype, suggesting that each of the five protein domains, as well as the interdomain regions, are all relevant for the physiological protein function.

Patients carrying large deletions, including the entire *CHD2* gene and contiguous genes, were reported to be more severely affected with an earlier age of seizure onset, multiple seizure types, episodes of SE, MRI abnormalities (specifically cerebellar vermis hypoplasia), and dysmorphogenetic features (specifically mild facial dysmorphisms, microcephaly, short stature, hypotonia, congenital hypothyroidism, bicuspid aortic valve, micropenis, single palmar creases) (Li et al., 2008; Veredice et al., 2009; Lund et al., 2013) (Supplementary Table 2). Our literature review highlighted that deletions smaller than 1 Mb or partially including *CHD2* do not necessarily produce more severe phenotypes compared with patients carrying frameshift or missense pathogenic or likely pathogenic variants. Therefore, we hypothesize that the involvement of contiguous genes contributes to determine the phenotypic severity in patients with large deletions including *CHD2*.

The clinical presentation of patients harbouring missense *CHD2* pathogenic or likely pathogenic variants is mostly indistinguishable from that of patients with frameshift/nonsense or splicing pathogenic variants.

A different clinical presentation characterised by adult-onset pharmacoresistant epilepsy and mild intellectual disability was observed in Patient 89, carrying a *de novo* p.Trp1261Ser missense likely

pathogenic variant. This variant is in the interdomain region between the SNF2-like helicase/ATPase domain and the DEDX-helicase domain and determines a semi-conservative amino acid substitution, which may impact on the secondary protein structure. The p.Trp1261Ser variant is absent in the gnomAD database, occurs in a position that is conserved across species, and is predicted to be probably damaging by *in silico* analyses (https://varsome.com/). For these reasons, this variant was classified as pathogenic according to the ACMG guidelines. A likely pathogenic missense variant, involving the same 1261 aminoacidic residue (p.Trp1261Leu) was recently reported in a child with developmental delay, language impairment and generalised epilepsy (Ziats et al. 2020), confirming the pathogenicity of p.Trp1261Ser whose association with adult-onset pharmacoresistant epilepsy expands the phenotype presentation of patients with *CHD2* pathogenic variants.

Familial cases originated from parents who were either mildly affected (Galizia et al., 2015; Chen et al., 2020) or healthy (Petersen et al., 2018; Chen et al., 2020), or from gonadal mosaicism (Pinto et al., 2014; Lebrun et al., 2017). Reports of identical phenotypes in monozygotic twins, and of mild differences in dizygotic twins, or of discordant phenotypes in siblings carrying the same deletion or missense likely pathogenic variant, emphasise the modulatory role of epigenetic or additional genetic factors (Pinto et al., 2014; Lebrun et al., 2017; Jiao et al., 2019; Chen et al., 2020). For example, Petersen et al firstly reported an inherited pathogenic *CHD2* frameshift variant in a girl with generalised epilepsy with multiple seizure types and episodes of SE, developmental delay, mild intellectual disability and ADHD and her mother with a milder form of generalised epilepsy, normal cognitive functioning, ADHD and bipolar disorder (#53 and #54) (Petersen et al 2018). Chen et al. described a couple of dizygotic twins (#71 and #72) with a paternally inherited missense likely pathogenic variant, located in an interdomain region (Chen et al 2020). While their father only had febrile seizures, the twins presented seizure-onset at 14 months and 8 months respectively, with both febrile seizures and afebrile generalised tonic-clonic seizures. It is reported that one sibling (#71) had more prolonged seizures than the other, including episodes of febrile SE (Chen et al., 2020). Finally, two brothers (#40 and #41) with a germline missense pathogenic variant in the SNF helicase/ATPase domain were reported to share the same cognitive, behavioural, and relational profile characterised by mild intellectual disability, aggressive behaviour, and autism but only one of them had epilepsy (#40) (Lebrun et al., 2017). A similar phenotypic discrepancy was reported in two brothers with a germline 83 kb deletion (Pinto et al., 2014).

Patients #85 and #92 harbour the same missense likely pathogenic variant p.Arg699Trp, but they have a different phenotype. Patient #85 had an epileptic encephalopathy with seizure-onset at 18 months, with evidence of motor regression two months later, with subsequent evidence of severe

intellectual disability, absence of language and scoliosis. Patient #92 had a milder clinical presentation with pharmacoresponsive epilepsy, classified as epilepsy with myoclonic-atonic seizure (EMAS), with seizure-onset at 2 years, language delay and attention deficit; motor development was normal and there was no scoliosis.

Another recurrent likely pathogenic variant was p.Arg900Gly in patients #86 and #96. Patient #86, female, had normal motor development but developed a defective expressive language, moderate cognitive impairment, and ADHD. Seizure onset was earlier compared with patient #96, around the age of three years, but only absences with eyelid myoclonia were present, with no photoparoxysmal response, and seizures responded to treatment with valproate and ethosuximide. Patient #96, male, had a history of severe global developmental delay, autistic features, and aggressive behaviour. At 8 years, he manifested absences with eyelid myoclonia and later developed multiple seizure types. His level of cognitive impairment was severe.

Overall, the combination of these observations suggests that the same genotype can produce different clinical phenotypes both in families and unrelated patients, with considerable variability of age at seizure onset, response to pharmacological treatment, photosensitivity, motor impairment, behavioural difficulties, language, and cognitive skills.

The human CHD2 protein (http://pfam.xfam.org/protein/O14647) is composed by 1828 amino acid residues. It contains two chromodomains located on N-terminal region, the SNF2-related N-terminal domain, the Helicase conserved C-terminal domain, the CHD 1/2 SANT-Helical linker 1 domain and a domain of unknown function (DUF 4208). The DUF 4208 is present in numerous DNA-binding protein so it is considered a putative DNA-binding region. The C-terminus of CHD2 also associates with a poly ADP-ribose (PAR) binding region that is involved in DNA damage repair (Luijsterburg et al., 2016). Experiments using CHD2 deletion constructs demonstrated that the N-terminus of CHD2, containing the two chromodomains, increases both DNA-binding and ATPase activities, and is required for the chromatin remodelling activity of the protein (Liu et al., 2015). The C-terminal DNA binding domain senses double stranded DNA and enhances the chromatin remodelling activity of CHD2 (Liu et al., 2015). The majority of reported patients harbour truncating *CHD2* pathogenic variants (Suls et al., 2013). Phenotypes associated with missense pathogenic or likely pathogenic variants in these N-terminal and C-terminal domains or with truncating pathogenic variants are indistinguishable. These observations suggest that haploinsufficiency is the main pathogenic mechanism underlying *CHD2*-associated epilepsy. The functional relevance of CHD2 haploinsufficiency has been explored in mice and zebrafish. Mice homozygous for a partial deletion of the DNA binding domain (DBD) at the Chd2 C-terminus exhibit growth retardation during late embryogenesis and perinatal lethality (Marfella et al., 2007).

Heterozygous mice for the partial DBD deletion exhibit heart, muscle, lung, liver, kidney, spleen and bone abnormalities; brain defects were not reported (Lamar and Carvill, 2018). A new *Chd2* mutant mouse line harbouring a exon 3 deletion, developed by Kim et al. exhibited altered neurogenesis in the embryonic forebrain with reduction in GABAergic inhibitory interneuron progenitors as well as defects in synaptic transmission, cortical synchrony and hippocampal-dependent memory behaviour (Kim et al., 2018). Changes in excitatory and inhibitory synaptic functions observed in these mice did not result in clinical or electrographic seizures during prolonged (i.e., 7 days) EEG monitoring. *Chd2*-knockdown zebrafish with targeted morpholino antisense oligomers (MO) (Suls et al., 2013; Galizia et al., 2015) exhibited multiple developmental abnormalities, altered locomotor activity and seizure-like behaviour, whose epileptic nature was confirmed by field-potential recordings. *Chd2*-knockdown zebrafish larvae displayed a markedly enhanced photosensitivity compared with their mild innate response (Galizia et al., 2015). Overall, these data suggest that CHD2 regulates neurodevelopmental genes during differentiation, supporting epileptogenesis in patients with CHD2 haploinsufficiency. Understanding how pathogenic variants in chromatin remodelling genes impact brain function may reveal new opportunities for targeted therapies.

Conclusion

CHD2-associated phenotype encompasses a wide spectrum of neurodevelopmental disorders, including early-onset global developmental delay with predominant language impairment, attention deficit, hyperactivity, autistic features, and behavioural problems. Epilepsy is the most frequent clinical presentation, in particular DEE with multiple seizure types, mainly generalised. Most of the molecular defects occur *de novo* and mainly consist of missense or truncating (frameshift, nonsense, splicing, deletions) pathogenic or likely pathogenic variants with no hotspots.

We expand the knowledge of *CHD2*-related disorders with the description of 12 novel pathogenic or likely pathogenic variants carried by 18 unrelated patients, one of those with a new phenotype consisting of adult-onset pharmacoresistant epilepsy.

We also reported one patient with a new phenotype consisting of adult-onset pharmacoresistant epilepsy. This observation confirms the importance of genetic testing in patients with epilepsy and neurodevelopmental disorders, even in adults with non-syndromic epilepsies when an aetiological diagnosis is lacking.

We found a wide variant spectrum associated with an equally wide range of heterogeneous phenotypes. Potential modulation by coexisting pathogenic variants in other genes or other factors,

such as the action of noncoding RNA genes, are likely to act as modifiers of the phenotype. Haploinsufficiency is the main molecular mechanism implicated in *CHD2*-related disorders, and approaches for increasing CHD2 levels may have therapeutic impact (Rom et al., 2019).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

None.

Author contribution statement

B De Maria contributed to data collection and analysis, and writing of the manuscript.

S Balestrini contributed to interpretation of the results and writing of the manuscript.

D Mei contributed to data analysis and creation of the Figures.

F Melani, S Pellacani, T Pisano, A Rosati, GM Scaturro, L Giordano, G Cantalupo, E Fontana, C Zammarchi, E Said, V Leuzzi, M Mastrangelo, and S Galosi, contributed to data collection and analysis.

Elena Parrini and Renzo Guerrini contributed to the study conceptualisation, data analysis and critical review of the manuscript.

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Table legends

Table 1. Genetic and clinical data of the patients reported in this study.

Table 2. Neurodevelopmental or psychiatric comorbidities, reported in 68% of patients with available data.

Supplementary Table 1. List of the 159 genes associated with epilepsy included in the next-generation sequencing (NGS) panel that we applied to 17/18 of our newly reported patients.

Supplementary Table 2. Genetic and clinical data of the previously reported patients.

Figure legends

Figure 1. Flow diagram illustrating the literature review process. Illustration of the stepwise approach used for inclusion of the relevant literature, including screening of genetic data followed by review of the available phenotypic information. Genetic inclusion criteria were gross deletions or duplications involving the *CHD2* gene, and missense or truncating variants classified as pathogenic or likely pathogenic according to the guidelines of the American College of Medical Genetics and Genomics (ACMG). We excluded those patients harbouring variants of uncertain significance (VOUS). We also excluded one paper not reporting the exact *CHD2* pathogenic variants. Of the patients with pathogenic or likely pathogenic variants meeting these genetic criteria (n=103), we included those with available information on neurological phenotype, including either the epilepsy type (n=76) or, for those without epilepsy, at least two of the following clinical items: motor development, speech development, cognitive functioning, presence/absence of attention deficit/hyperactivity, deficits in communication, social and learning skills (n=8).

Figure 2. Distribution of *CHD2* pathogenic or likely pathogenic variants identified in patients with epilepsy and neurodevelopmental disorders reported in literature and in our cohort

Top panel: *CHD2* pathogenic or likely pathogenic variants identified in our series of 102 new and published patients. Frameshift and nonsense pathogenic variants reported in literature are represented in red vertical lines, frameshift and nonsense pathogenic variants identified in our cohort are represented in red vertical lollipops, missense pathogenic or likely pathogenic variants reported in literature are represented in blue vertical lines, missense likely pathogenic variants identified in our cohort are represented in blue vertical lollipops

Bottom panel: missense *CHD2* variants present more than twice in the GnomAD dataset are represented in grey vertical lollipops.